Gene Therapy Project Summary

Preliminary Study

1 Encapsulation of ß-gal coding DNA linear and supercoiled in a PLA blend

- Materials: 1 g PLA (300K) dissolved in 5 ml of methylene chloride

  2 g PLA (2K) dissolved in 5 ml of methylene chloride

  ß-gal plasmid (1-2 mg/ml), diluted 1: 5

- Methods: The two solutions were mixed(no phase separation observed).

  5 drops of Span 85 was mixed into this solution.

  The resulting mixture was aliquoted into glass vials(2 ml/vial).

  In each glass vial, 100 µl of DNA(20 µg-40µg) was added.

  The glass vials were left in the refrigerator for two days and lyophilized.

- Note: After addition of the DNA solution, the polymer blend precipitated quickly and droplets of DNA were visible under optical microscopy.

Implantation of DNA/PLA pellets

- Sterilization: EtOh 5 min
  PBS-P/S 5 min

- Surgery: Each rat received linear DNA into the left leg and supercoiled DNA into the right. Implants were inserted into incised muscle - either the vastus or the hamstring. The muscle was sutured back together and then the skin.

Rat ID	Implant Duration	
R1	11/6 - 11/20/91	*2 weeks*
R2	11/6 - 3/6/92	*4 months*
R116	8/19 - 9/8/93	*3 weeks*

- Explant: Rats were perfused with PBS/heparin followed by 3% paraformaldehyde and 0.2% glutaraldehyde in PBS. Post-fix with 3% paraformaldehyde followed by 15% sucrose/PBS. Excised muscles were cut with a cryostat and stained with X-Gal.